REPRODUCTIVE SUCCESS IN DUNGENESS CRAB *(CANCER MAGISTER)* DURING LONG-TERM EXPOSURES TO OILCONTAMINATED SEDIMENTS

by

John F. Karinen, Stanley D. Rice and Malin M. Babcock

NOAA, National Marine Fisheries Service
Northwest and Alaska Fisheries Center
Auke Bay Laboratory
P.O. BOX 210155
Auke Bay, Alaska 99821

Final Report
Outer Continental Shelf Environmental Assessment Program
Research Unit 650

April 1985

ACKNOWLEDGMENTS

We are indebted to the following persons for their assistance in this study. Sid Kern and Adam Moles did the dosing and mixing of oil sediments for both phases of this study. Patricia Arasmith staged most of the egg samples. Jessica Gharrett and David Logan prepared the tissues for hydrocarbon analyses; Jeffrey Short ran the tissue samples on the gas chromatography and did the sediment samples on the IR spectrophotometer. Mark Carls advised on the statistical analyses.

ABSTRACT

Dungeness crab *(Cancer magister)* habitat in the nearshore waters of Alaska may be at risk from oil pollution. Transport of crude oil and expanded exploratory drilling in Alaskan waters increase the likelihood of accidental contamination of coastal sediments, and oil adsorbed by the sediments is likely to persist for years in subarctic and arctic waters. In contrast to other commercially important Alaskan crabs, Dungeness crabs completely bury themselves, particularly when spawning and incubating their eggs. Therefore, their eggs are in direct contact with sediments for extended periods and are vulnerable to any xenobiotics contaminating the habitat.

In Phase 1 of this study, we exposed adult female crabs to three dose levels of oiled sediments for one complete reproductive cycle (10 months) to determine effects on survival, uptake of hydrocarbons, hatching success, and viability of larvae. Each exposure tank, supplied with clean, flowing seawater, held nine female crabs on 15 cm of sediment with O, 1.2, 3.7, or 8.6 μl Cook Inlet crude oil per gram of sediment. The doses and controls were run in triplicate. The crabs were monitored daily for survival and behavior. Hatching began in mid-April, and larvae were captured in a trap attached to the outlet of each tank. At three different times during the hatching period, we held larvae, in triplicate from each dose, in tubes to determine viability.

Dosed crabs produced significantly ($0.025 < P < 0.05$) lower numbers of larvae than control crabs. Control crabs produced a mean of 368,700 larvae/crab, and the low, mid, and high doses produced 225,500, 303,900, and 268,100 larvae/crab, respectively. Larvae from the high-dose tanks survived for significantly ($P < 0.005$) shorter periods (3. 1 days) than larvae from the control tanks and low- and mid-dose tanks (5.3 days). Eggs from crabs in the high-dose tanks had significantly elevated levels of aromatic and aliphatic hydrocarbons, compared with eggs from crabs in control tanks.

In Phase 2, a subsequent 4-month experiment, we studied the effects of oiled-sediment exposures on mating and molting. Some of the females used in Phase 1 were continued in the Phase 2 exposures. The experimental tanks contained old (held over from Phase 1) control, old mid-dose, old high-dose, fresh high-dose, and new control sediments. Female crabs from Phase 1 exposures and previously unexposed male crabs were used in the old sediment tanks. Previously unexposed male and female crabs were placed on new sediments. All experimental conditions were triplicate except for the new control tank.

Clasping behavior and successful molting were low in all old-dose experimental conditions, and completely absent in crabs in the new high-dose tanks, but occurred in 75% of the pairs in the new control tank. The low rate of clasping and molting in all old,

14-month exposures was probably caused by a combination of confinement, diet, and oil. The briefer, 4-month exposure to freshly oiled sediment completely inhibited clasping behavior and molting.

Fifty-five percent of the female crabs that had not molted subsequently spawned and established fertile clutches. At the termination of the exposures, examination revealed the presence of fresh sperm in the spermathecas of over 90% of the females, indicating these females had copulated in the hard-shelled condition. We believe that spawning without molting is an adaptive response for using energy reserves that may have been depleted by a combination of stressors.

Exposure to oiled sediments results in lowered reproductive activity in Dungeness crabs, and the larvae produced are not as robust, as indicated by significantly shorter survival times, compared with control larvae. Therefore, over a period of time, the presence of oil in the habitat substrate may lower population densities.

TABLE OF CONTENTS

INTRODUCTION

The benthos is the ultimate sink for hydrocarbons in the marine environment. Oil may be introduced to the benthos directly in submarine spills, such as the *Ixtoc I* spill, or indirectly from surface spills (Jordan and Payne 1980; Karinen 1980; MacKay et al. 1981). Once hydrocarbons reach the sediments, they may persist for years (Burns and Teal 1979; Haines and Atlas 1982). Krebs and Burns (1977) followed the West Falmouth oil spill and found deleterious effects on *Uca pugnax* populations for as long as 7 years after the spill.

The potential for oil pollution to occur in productive benthic sediments in Alaska is great. Cook Inlet has had producing oil wells since 1958, and tankers have been transporting crude oil from the North Slope through Alaskan waters since 1977. In more recent years, active exploratory drilling has been expanded to several nearshore and offshore areas in Alaskan waters.

At some time during their life cycles, many marine organisms of commercial importance reside on, bury in, or have other interaction with sediments. Invertebrate eggs, larvae, juveniles, and adults accumulate hydrocarbons during extended periods of exposure to oil (Brodersen et al. 1977; Rice et al. 1983). Eggs carried by crabs are in close proximity to sediments, and are buried in sediments when the female burrows. Dungeness crabs (Cancer *magister*), particularly ovigerous females, spend much of their time buried. This behavior increases the chance that hydrocarbons incorporated in sediments and in interstitial waters may enter the eggs or the body of the crab or both and affect development of internally or externally carried eggs. Hydrocarbons may affect reproduction directly, by killing developing eggs and larvae; or indirectly, by increasing overall energy demands and causing a consequent reduction in production of gonad material.

Previous studies at the Auke Bay Laboratory (Karinen et al. 1983; Rice et al. 1983) on oiled-sediment interaction with juvenile king crabs (*Paralithodes camtschatica*) and ovigerous Tanner crabs (*Chionoecetes bairdi*) indicated no effects of oiled sediments on survival and reproduction but showed significant tissue uptake of hydrocarbons. Juvenile king crabs and adult Tanner crabs rarely bury themselves, so the whole body and developing eggs do not directly contact the sediments. In contrast, developing eggs of Dungeness crabs maybe in direct contact with sediments for the entire incubation period (about 6 months).

Our objectives in Phase 1 of this study were to determine the effects of long-term (10-month) exposures to oil-contaminated sediments on survival, hatching success, and viability of larvae; and in Phase 2, to determine effects of 4- and 14-month exposures to oil-contaminated sediments on molting and mating.

METHODS

Phase 1 of the oil-sediment exposures was started with mature female Dungeness crabs *(Cancer magister)* in August 1983 and ended in early July 1984. Phase 2 of these exposures, using both female and male crabs, began in July 1984 and terminated in early November 1984. Some of the females from Phase 1 were used in the Phase 2 exposures.

Phase 1

The crabs were exposed in 12 loosely covered Living Stream tanks (208x60x60 cm) consisting of triplicate controls and doses. Each tank had seawater flowing through it at 3 liters/rein, allowing for a complete exchange of water every 2.2 hours. Temperature (Fig. 1), light, and oxygen levels were not controlled but approximated natural environmental conditions.

We obtained sediment from the Auke Bay Recreation Area, near Juneau, Alaska, using a 7-inch by 2-foot dredge, This area is relatively pristine and is known to contain Dungeness crabs. Upon collection, the macrobenthos was removed, and the sediment was frozen in 5-gal buckets until needed. Four days before dosing, the sediment was allowed to thaw.

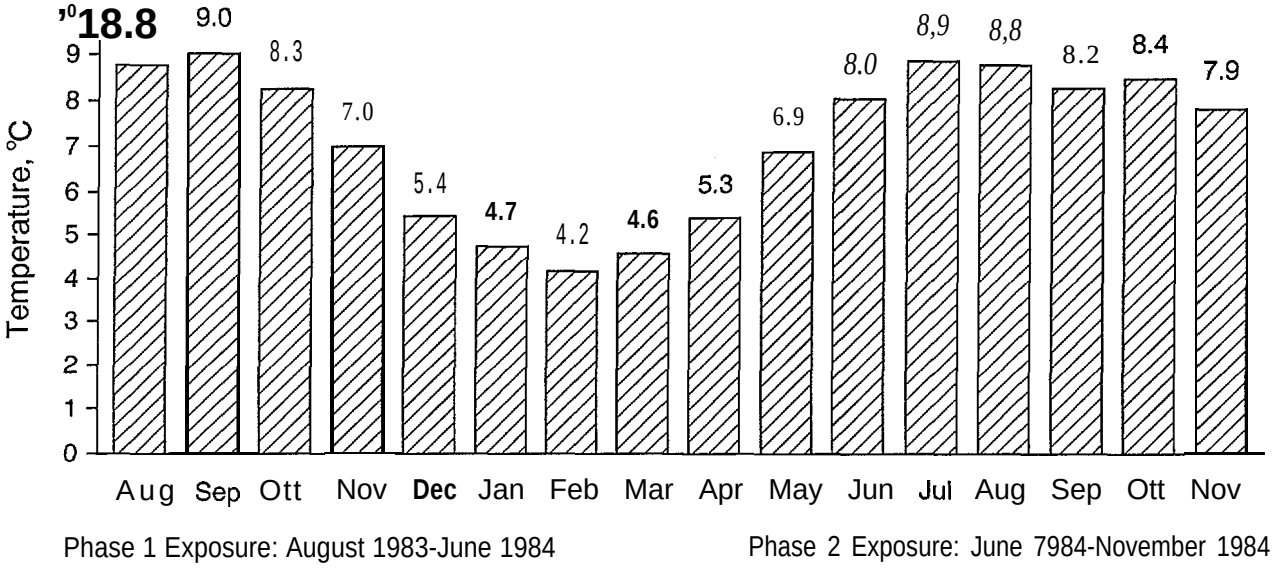

Figure 1.–Average seawater temperatures in Dungeness crab (Cancer *magister*) exposure tanks.

Nominal dosing levels were O (controls), 0.20, 0.80, and 2.00910 v/v Cook Inlet Crude oil/sediment. The 2% dose was the maximum that the sediment could retain and hold. These doses were chosen because, after initial settling and evaporation, they should have approximated hydrocarbon levels which might be expected in the environment. Sediment (90%), sand (109Io), and Cook Inlet crude oil in the appropriate amount were mixed in a portable cement mixer. Additional sand was used to prevent compaction that had occurred in a prior sediment exposure system. The particle composition of the sediment mixture was 11.6% gravel, 79.4910 sand, 5.3% silt, and 3.790 clay. (The sediment was analyzed by Battelle-Northwest Research Laboratory, Sequim, WA.) The mixture was then placed in the bottom of the exposure tanks to a depth of about 15 cm. Selection of dosed tanks was random. We let the tanks condition with clean seawater flowing through them for 4 days, before introducing the test animals.

Recently molted, barren, but presumably mated, mature female Dungeness crabs were obtained by pot fishing or by divers in the Juneau or Sitka areas and held at the Auke Bay Laboratory in clean, flowing seawater at least 1 week prior to the exposures. Randomly selected, healthy crabs were examined for missing limbs and general condition, weighed, and measured, and spines were clipped in binary code (for individual identification), before crabs were placed in the exposure tanks. Exposures began with eight crabs per tank, A ninth crab was added later for measurement of hydrocarbon uptake in several tissues. They were fed chopped herring until satiated one or two times per week.

The sediment was analyzed by infrared spectrophotometry (IR) in August and November 1983 and in May 1984. Sediment was sampled from three locations in each tank, and the samples were analyzed individually (nine analyses per dose). The August 1983 samples (after 4 days of dosing and before we added the crabs) were a composite top and bottom sample (sediments were homogeneous at that time). Subsequent samples were divided into top and bottom portions, the top samples representing the top 2 cm of a 10-cm core. An aqueous slurry of each sample was extracted into Freon, and its absorbance at 2930 nm was measured by IR.

We monitored the crabs daily for survival, egg extrusion, and behavior (particularly burying activity). The location of each crab was noted, and a code of 1 (on top), 2 (partially buried), or 3 (completely buried) was assigned to indicate the depth of each crab. General activity levels and unusual behavior were also noted. Disturbance and handling of individual crabs were kept to a minimum.

At bimonthly intervals, egg samples were taken and fixed in Gilson's fluid for later examination. We used an adaptation of Boolootian's (1959) scheme of egg development stages to assess embryonic progress (see Appendix).

Starting in mid-April 1984, we measured hatching success and larval viabilit y. Zoeae were captured in 10-inch circular, fine-mesh net traps that were attached to each tank outlet. Daily production from each tank was filtered and dried at 60°C to a constant weight (usually 24 hours). The dried weight was then divided by the number of gravid females in that tank to give production/crab. Individual larval weight was calculated by filtering a small number (200-500) of larvae, drying as above, weighing, and counting them. There were six replicates. The dry weight of a single larva was calculated to be 3.2×10^{-5}. We determined the number of larvae hatched by dividing the production weight by the weight of one larva. In the ANOVA analysis of the production data, we used a Y^2 transformation of larval production to overcome variance heterogeneity. Variance increased with decreased production and increased dose level,

Hatching was essentially completed by early July 1984, when the Phase 2 exposures began.

Larval viability was tested three different times during the hatching period. We tested viability by measuring survival time of unfed larvae. Twelve to fifteen larvae from each dose were carefully pipetted from a swarm in an exposure tank to a test tube (2.5 x 12.0 cm) filled with seawater (50 ml). Triplicate tubes were run for each dose. All tubes were placed in a rack in a flowing seawater bath. The temperature of the water was not controlled; it ranged from 5.60 C in April to 8.7 *C in June. We examined the tubes daily for activity and survival and assigned a category reflecting each individual's activity pattern:

Swimming: in the water column.

On Bottom: alive and responsive to stimuli.

Opaque: dead.

In January 1984 and again in June 1984, we sacrificed one crab from each tank (three crabs per dose) for hydrocarbon analysis of various tissues. Gills, eggs, ovarian tissue, digestive gland (hepatopancreas), and muscle were frozen for later analysis of aromatic hydrocarbons. Samples were weighed, heated, digested in 10N NaOH, and extracted in hexane. The hexane extract was run through a silica gel column to separate the aliphatic fraction from the aromatic fraction. These fractions were then concentrated and analyzed by capillary column gas chromatography.

Standard statistical analyses, including means, 95% confidence intervals, and analyses of variance, were done where appropriate.

Phase 2

This phase of the study began in July 1984 and ended in November 1984. Our objectives were to determine the effects of exposure to oiled sediment on molting and mating.

Because the dose levels of oil in the sediment were stable during the Phase 1 long-term exposures (see Fig. 2), we used the three old (held over from Phase 1) control, three high-dose (2.0%), and three mid-dose (0.80910) tanks as part of the experimental design. In addition, we had one new control tank and three new, freshly oiled, high-dose (2%) tanks. Females in the holdover tanks were retained in their respective tanks insofar as possible for this phase. All male crabs and new females for the four new tanks were obtained from local waters 2 weeks before the beginning of these exposures.

The tanks were divided into four sections, each containing one male and one female, for a total of 52 pairs. We monitored the crabs daily, as described for Phase 1, paying particular attention to evidence of clasping which occurs prior to molting.

At the termination of the exposures in November, three females from each tank were sacrificed and tissues were frozen for aromatic hydrocarbon analyses, as described above.

RESULTS

Physical Parameters

Temperatures ranged from a low of 4.00 C in February 1984, during the Phase 1 exposures, to a high of 11.2 °C in August 1984, during the Phase 2 exposures (Fig. 1). Although the temperature varied as much as 0.8°C from day to day, variation among tanks on any given day was <0.30 C. Oxygen levels in the seawater throughout the exposures approached saturation level, and salinity ranged from 28 to 30 ppt.

Sediment Hydrocarbons

The levels of oil adsorbed by sediment below 2 cm remained remarkably constant throughout the 10-month exposure period (Fig. 2, Table 1). Hydrocarbon levels in May 1984 (8.38 μl/g, high dose) were very similar to initial levels in August 1983 (8.86 μl/g, high dose), 4 days after dosing and before introduction of the crabs. In contrast,

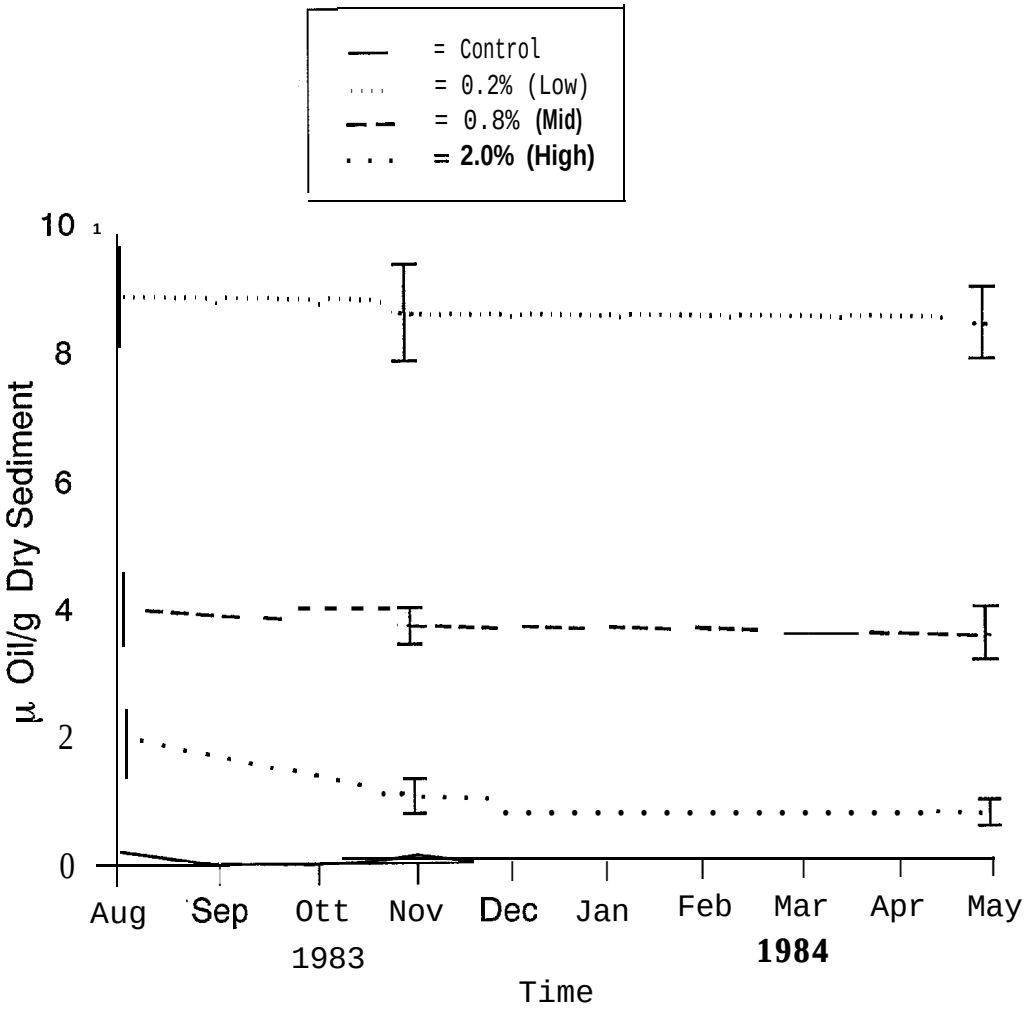

Figure 2.—Total sediment hydrocarbons in Dungeness crab (Cancer *magister*) exposure tanks. The November 1983 and May 1984 samples were taken below 2 cm; the August 1983 sample is a composite. N =9, (Vertical lines= Standard Error.)

Table 1.—Aromatic hydrocarbons in sediments as measured by infrared spectrophotometry (pi/g oil/sediment). The top samples included the upper 2 cm of sediment; the bottom samples, 2-10 cm. Samples taken in August 1983 were a composite depth sample. Figures are means of triplicate samples within tanks and between tanks of the same dosing level (N= 9). Two-day ANOVA shows significant separation of values.

Dosing level	Top/Bottom	Bottom		Top	
	Aug. 1983	Nov. 1983	May 1984	Nov. 1983	May 1984
Control	0.14	0.13	0.01	0.17	0.05
0.20%	1.93	1.03	0.77	0.47	0.15
0.80%	3.98	3.70	3.55	0.95	0.28
2.00%	8.86	8.53	8.38	1.62	0.78

hydrocarbon levels in the upper 2 cm of sediment decreased with time; levels in May 1984, 9 months after initial dosing, were only 7-15'%0 of the original values (Table 1). Even after several months of exposure, oil slicks were occasionally noted on the water surface of the mid- and high-dose tanks. After 2 months, analysis of the water above the sediments indicated there were no detectable hydrocarbons present.

Phase 1

Survival

There were some mortalities during the 10-month test, but they were not related to oil exposure. Overall survival was 86%; survival among doses varied from 93% in the mid-dose to 82% in the low dose (Fig. 3), but these differences were not statistically significant. Cause of death was usually unknown.

Behavior

The crabs were generally inactive during the day except when feeding; then they would move about the tank. The crabs in the high-dose tanks were hyperactive during the first month of the exposures. Frequency of burying increased after this time and averaged 40–70% in a tank on any given day, with differences being unrelated to dosing levels. Oil in the sediment did not inhibit burying behavior at any of the dosing levels after one month.

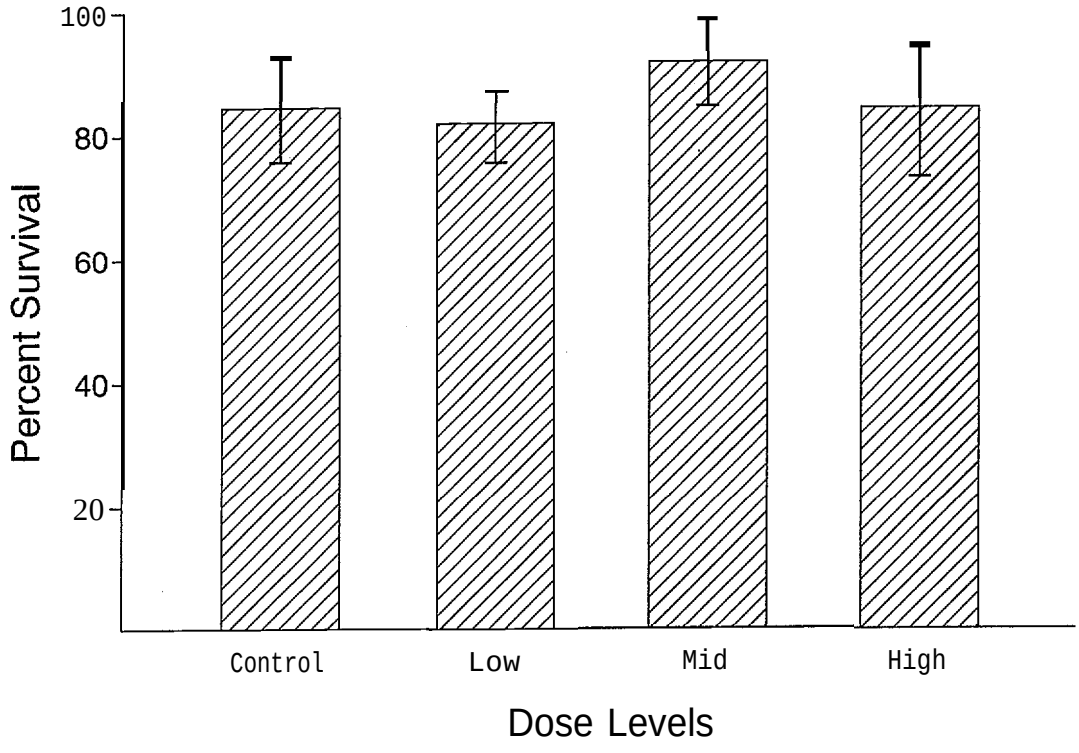

Figure 3.–Survival of female Dungeness crabs *(Cancer magister)* exposed to oiled sediments, August 1983 through June 1984. (Vertical lines = Standard Error.)

Egg Extrusion

Eighty percent of all crabs produced eggs with no significant differences among doses (Table 2). Female Dungeness crabs bury themselves during egg extrusion, and we wanted to disturb them as little as possible; therefore, it was difficult to determine when actual extrusion occurred. Successful establishment of clutches was observed in mid-September (1 month after exposures began) and continued through January 1984. Only five crabs produced clutches which we rated as less than half full (Table 2); all other clutches were assigned a three-fourths full or full rating. None of the crabs lost an entire clutch before hatching began in April 1984, but three crabs lost part of their clutches. Two of these had *Carcinomertes* sp. infestations in the egg masses. Obvious infestations of *Carcinomertes* sp. and nematodes occurred randomly in 10% of all crabs.

Table 2.—Reproductive data for Dungeness crabs *(Cancer magister)* exposed to oiled sediments, August 1983 through June 1984.

Dose level (µl/g)	Mean % gravid (± S. E.)	Number with clutches < ⅛ full	Number with partial clutch loss	Mean dry weight (g) of clutch (± S. E.)	Mean number of larvae
Control	82 ± 10	1	0	11.43 ± 0.44	368,710
Low: 1.24	78 ± 6	2	2	6.99 ± 0.24"	225,480
Mid: 3.74	79 ± 5	1	0	9.42 ± 1.02"	303,870
High: 8.59	79 ± 15	1	1	8.31 ± 1.53*	268,060

* $0.025 < P < 0.05$

Microscopic examination of the eggs for developmental stages revealed a uniformly high (>97%) fertilization success. Developmental stages within an individual clutch were uniform, but there was variation among crabs within a tank.

Larval Production

Control crabs produced significantly ($0.025 < P < 0.05$) more larvae than the dosed crabs (Table 2); however, the reduced production among the dosed crabs was not linear. Control crabs hatched 368,700 larvae/crab, and crabs in the low-, mid-, and high-dose tanks hatched 225,500, 303,900, and 268,100 larvae/crab, respectively. Hatching started in mid-April and continued through the end of June. Time of hatching in the various tanks was unrelated to dosing.

Larval Viability

Larvae from crabs that were in the high-dose tanks lived for significantly shorter periods than larvae from control and lower-dose tanks (Fig. 4). Mean survival time of 50% of the larvae from the control, low-dose, and mid-dose tanks was 5.3 days, while 50% of the larvae from the high-dose tanks lived an average of only 3.1 days. Larvae hatching in the high-dose tanks behaved differently than larvae in the control, low-dose, and mid-dose tanks; they did not form the compact, tightly massed swarms observed in the control and lower-dose tanks.

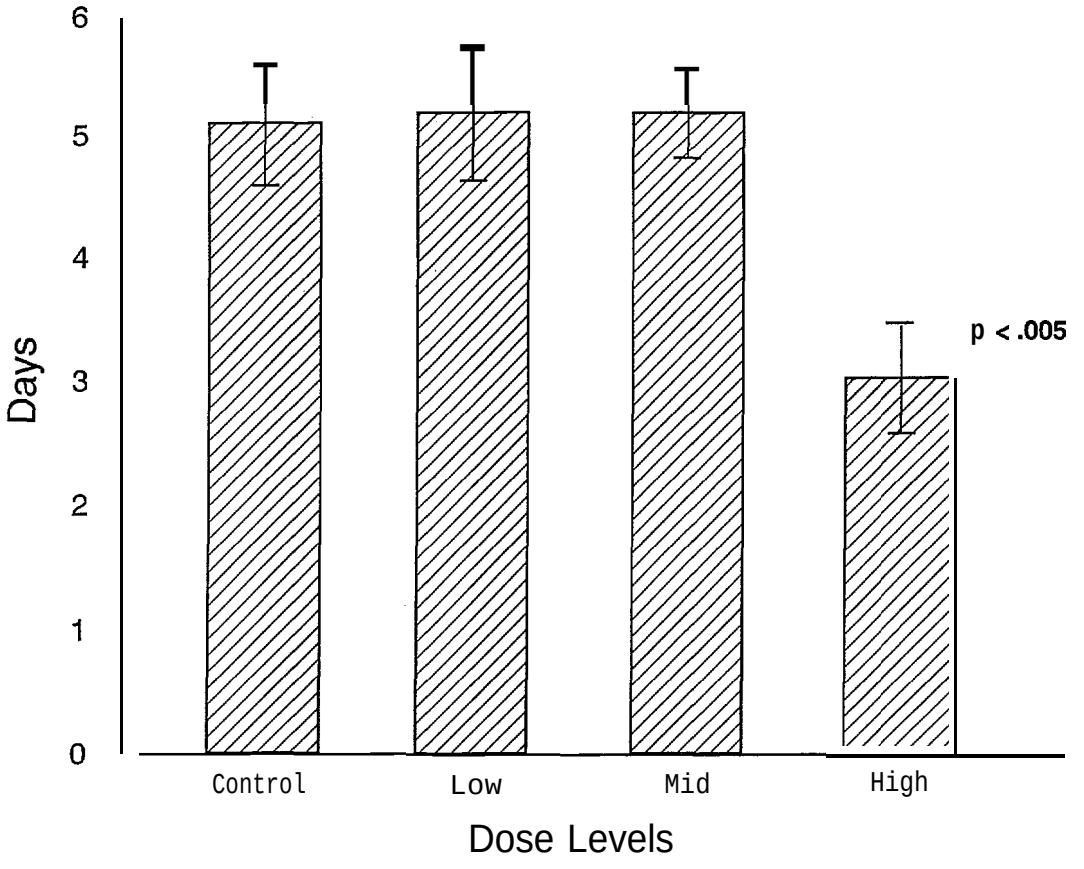

Figure 4.—Mean survival time of 50% of the larvae hatched from Dungeness crabs *(Cancer magister)* exposed to oil sediments. (Vertical lines= Standard Error.)

Hydrocarbon Uptake

Incubating eggs and the digestive gland tissue taken from crabs in the high dose showed significantly greater uptake of hydrocarbons than those from the control crabs (Table 3, Fig. 5). Samples were taken after 4, 10, and 14 months of exposure, but there was no correlation of uptake with time. Ovarian, muscle, and gill tissues showed no differences between control and dosed animals.

Phase 2

This phase began the first week in June and ended the first week in November. Survival of the crabs in both the new and the old high doses was significantly lower than that of control crabs (Table 4).

Table 3 .—Mean hydrocarbon levels in tissues from Dungeness crabs *(Cancer magister)* exposed (4-14 months) to oiled sediments (ppm or μg/g).

Tissue	Dose	N	Mono-aromatics	Di-aromatics	Poly-aromatics	Aliphatics	Total hydro-carbons
Eggs	Control	4	1,0	0,2	0.9	1.8	3.9
	High (8.59 μl/g)	5	9.8**	13.1***	6.1**	29.0***	32.7**
Digestive gland	Control	13	2.2	1.2	32.2	35.6	37.3 n.s.
	High	11	14. 1*	5.9**	34.1**	52.8*	51.3
Ovary	Control	11	0.7 n.s.	0.1 n.s.	0.8 n.s.	1.6 n.s.	9.9 n.s.
	High	12	0.8	0.2	0.8	1.8	11.8
Muscle	Control	6	0.8 n.s.	0.1 n.s.	0.2 n.s.	1.2 n.s.	0.6 n.s.
	High	6	0.4	0.1	0.1	0.5	0.8
Gill	Control	6	0.5 n.s.	0.2 n.s.	0.7 n.s.	1.4 n.s	1.5 n.s
	High	7	0.3	0.2	0.8	1.3	6.2

** 0.01 $< P <$ 0.025
** 0.025 $< P <$ 0.05
* 0.05 $< P <$ 0.10
n.s. Not significant

Table 4.—Summary of data from female Dungeness crabs (Cancer *magister*) exposed to oiled sediments during the mating and molting phase, July to November 1984.

Dose	Number of females	Females survived (%)	Number of clasping pairs	Number molting	Number with eggs (no molt)	Fertile?
New Control	4	100	3	3	1	yes
Old Control	12	83	2	2	6	yes
Old Mid Dose	13*	87	1	1	3	yes
Old High Dose	13*	70	2	1	7	yes
New High Dose	15*	73	0	0	8	yes

* The crabs that died in July and early August were replaced with other females that had a similar history of exposure/nonexposure.

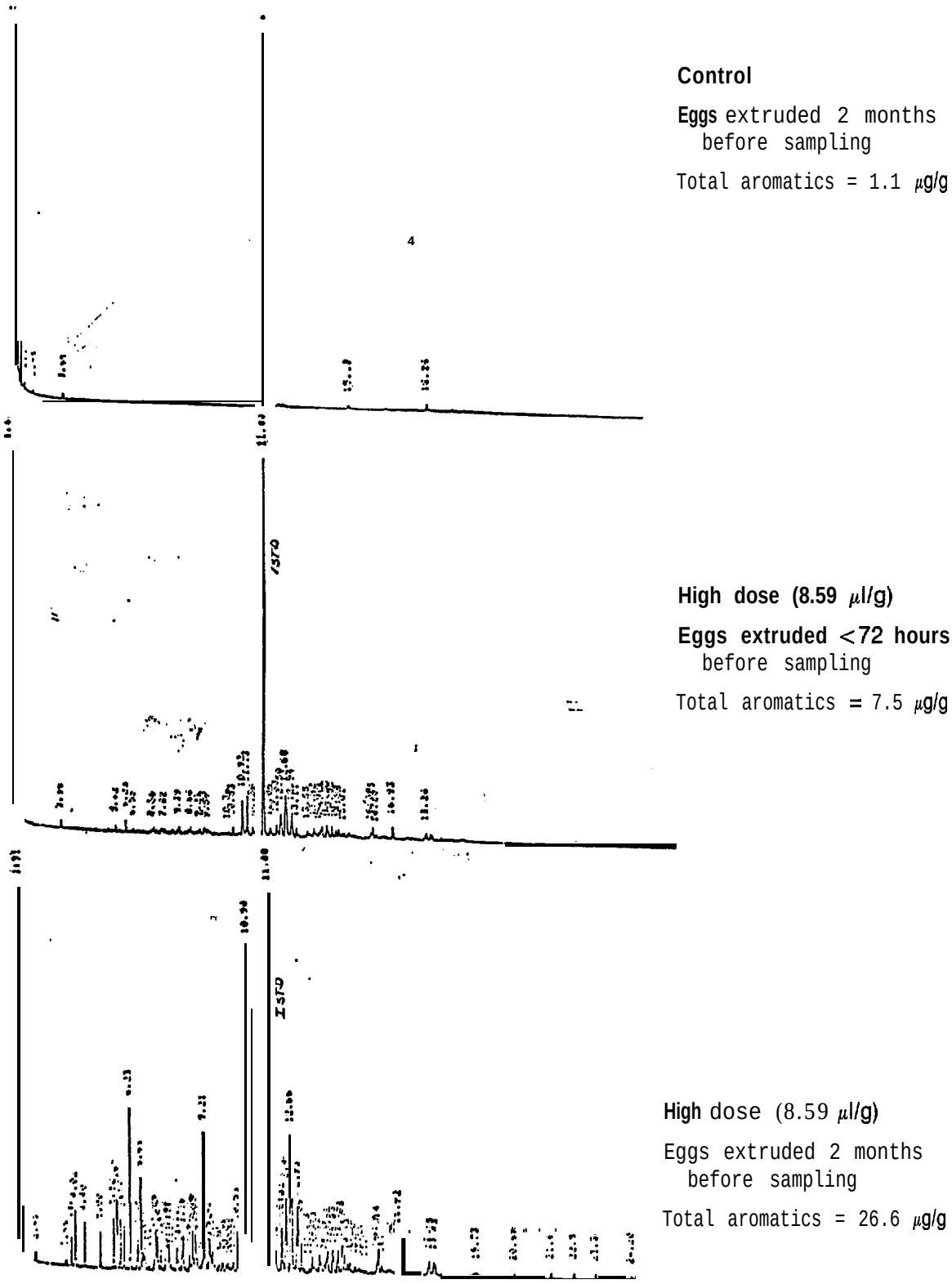

Figure 5 .—Gas chromatographic scans of aromatic hydrocarbons in eggs from Dungeness crabs (*Cancer magister*) that had been exposed to oiled sediments for 4 months. Eggs giving the middle scan were not in direct contact with the sediment as long as the eggs shown in the scan below. ISTD = Internal Standard.

Clasping behavior, a prelude to molting in the female, was observed in 8 of the 52 pairs. This behavior preceded actual molting by 3-15 days and occurred from mid-August through the end of the exposures in November. Five of the eight pairs were controls, and none of the pairs in the new high dose (-8.59 μl/g) exhibited any clasping or other mating behavior. Seven of the eight females successfully molted; one female in an old high dose died while molting (Table 4).

None of the females that had molted and presumably mated extruded new clutches by the termination of the exposures in November. However, 25 (55%) of the females that had not molted extruded eggs and successfully established fertile clutches (Table 4). Most of the clutches in the females that had already been exposed in Phase 1 were paler than either the first clutches or those of crabs exposed beginning in July 1984. This was undoubtedly due to the absence of carotenoid pigment in their diet.

Copulation was not observed in any of the pairs, but when crabs were sampled in November for hydrocarbon analyses, fresh sperm was observed in >90910 of the spermathecas, whether the crab had recently molted or not.

The hydrocarbon uptake data from the November 1984 sampling are included in the Phase 1 section.

DISCUSSION

Sediment Hydrocarbons

The persistence of oil in the sediments in temperate and arctic regions is fairly well documented (Krebs and Burns 1977). This was clearly corroborated in our exposures, which showed no significant losses of hydrocarbons from sediments below 2 cm over a 10-month period. Total hydrocarbons in the upper 2 cm did decline to 7-15970 of the original values. Rice et al. (1983) observed greater losses of hydrocarbons in the deeper (15%) sediments during a similar, but shorter, series of oiled-sediment exposures with juvenile king crabs. Because sediment is agitated to a depth of about 6 cm during burying activity of Dungeness crabs, a similar decline in these exposures was expected. The only difference in the sediments between the two experiments was the addition of 10% sand in our exposure to minimize compaction.

Oil slicks on the water surface were seen frequently in the mid- and high-dose tanks at the beginning of the exposures and were observed occasionally throughout the course of the tests, even though there were no detectable hydrocarbons in the water-soluble fraction (WSF) of oil. Burying activity would periodically agitate the sediment and release some hydrocarbons.

Total survival was 86%, with no differences among the experimental groups. A similar series of exposures with Tanner crab (*Chionoecetes bairdi*) that lasted 14 months showed 93% survival (Karinen et al. 1983).

In their natural environment, Dungeness crabs frequently bury themselves in the sediment. During the course of these experiments the crabs buried themselves and remained inactive during the day. Except at the beginning of the experiment, when the animals in the mid- and high-dose tanks were restless, the presence of oil in the sediments did not inhibit this behavior. Olla et al. (1981) did not detect any changes in overall activity levels of Dungeness crabs during their tests with oiled sediments. Similar restlessness at the beginning of the exposures was noted in Tanner crabs (Karinen et al. 1983). The greater activity levels of the crabs in the mid and high doses at the beginning of the exposures was probably due to concentrations being well within the range of the crabs' ability to detect them (Pearson et al. 1980). The gradual disappearance of hydrocarbons from the water column (none detectable after 2 months) coincided with burying behavior identical to that of crabs in the control tanks.

Dungeness crabs may detect and avoid areas of oiled sediments, but Olla et al. (1981) found mixed results in their avoidance tests with oiled sediments and Dungeness crabs. They found that crabs avoided the highly oiled sand but tended to prefer moderately and low-oiled sediments to clean sand. (Their experimental concentrations of oil in sediment were much less than ours: 2,508, 192, and 18 ppm vs. 8,590, 3,740, and 1,240 ppm. Both studies used IR spectrophotometer for analysis.)

The high incidence of successful establishment of clutches, rated mid to full, was virtually identical to that found in a previous study with Tanner crabs (Karinen et al. 1983).

Stage of development of eggs from crabs in the same tank was variable and probably reflects the extended period of egg extrusion and fertilization (September–January) and actual hatching (mid-April to the end of June). In contrast, the stage of eggs in the brood pouches of Tanner crabs was remarkably similar among individuals within the same tank. Hatching of Tanner crab larvae took place over a 4-week period, in contrast to the Dungeness hatching period of less than 10 weeks.

Reduction in total output of larvae per Dungeness crab in the dosed tanks compared to controls contrasted with the results of an earlier series of exposures done by Karinen et al. (1983) with Tanner crabs. There was no difference in amount of larvae hatched in dosed tanks versus controls, and no apparent effect on viability of dosed and control larvae.

However, dosed Tanner larvae swarmed less than controls, similar to behavior observed in Dungeness larvae. Our results also contrasted with those in a study by Ebert et al. (1975). They compared hatching success (expressed as viable larvae released) in individual crabs taken from San Francisco Bay (polluted area) with crabs taken near Eureka-Crescent City, California (a relatively pristine area). Although hatching success in crabs from the Eureka-Crescent City area was greater (90% vs. 80910), this difference was not statistically significant. They used larval counts from a total of only 15 animals, and high variability y seen in individual animals probably masked any difference in production from the two areas.

Shorter survival times for larvae from the high-dose tanks compared to control larvae indicate that the dosed larvae had lower energy reserves and were weakened. Viability tests done on Tanner crab larvae following hatching from similar exposure tests showed no difference between dosed and control larvae (Karinen et al. 1983).

Uptake of hydrocarbons in the egg masses of crabs exposed to the high dose is not surprising because of the close physical contact between the eggs and the oil present in the sediments, Direct uptake of hydrocarbons from the environment by brooding eggs is indicated by two factors. First, newly (<72 h) extruded eggs of a crab in the high dose showed uptake levels that were intermediate between controls and other high-dose eggs that had been extruded over 2 months (Fig. 5). Second, ovarian tissue, even from crabs that had been exposed to the high dose for 14 months, showed no significant differences over control animals (Table 3). An earlier study (unpublished data) showed no difference between aromatic hydrocarbon levels in Tanner crab eggs from control and high-dosed crabs, although there were higher levels of aliphatic hydrocarbons in eggs from dosed crabs.

Digestive gland tissue from Dungeness crabs exposed to the high dose had approximately 1.5 times the level of aromatic hydrocarbons found in controls. Increased levels of hydrocarbons were also found in the digestive gland in juvenile king crabs that were exposed to oiled sediments" (Rice et al. 1983). After 90 days of exposure to 2% oil-dosed sediment, aromatic and aliphatic hydrocarbon levels in exposed crabs were 60 and 130 times the levels in controls. Rice et al. (1983) also found increased levels of aromatic hydrocarbons in muscle tissue of juvenile king crabs exposed 90 days to 0.5 ppm WSF of Cook Inlet crude oil and increased levels of aliphatics in muscle from crabs exposed to 2% oiled sediment. They found sediment particles in the guts of the juvenile king crabs, indicating ingestion was a major avenue of uptake. We found virtually no uptake of either aromatic or aliphatic hydrocarbons in muscle tissue from Dungeness crabs exposed to our highest dose of oiled sediments.

The differences in reproductive response to oiled sediments between Dungeness and Tanner crab probably reflect differences in burying behavior of the two species. Brooding Tanner crab rarely bury, whereas gravid Dungeness crabs spend the majority of their time buried in' sediments, allowing close contact between adsorbed oil and developing eggs.

The results of this 10-month study suggest that reproduction in Dungeness crabs may be impacted by exposure to oiled sediments, as shown by reduced larval production and viability and as reflected in uptake of aromatic and aliphatic hydrocarbons in incubating eggs. Krebs and Burns (1977) found behavioral peculiarities and reduced population densities of the fiddler crab, *Uca pugnax*, 7 years after the West Falmouth oil spill. So it is not surprising to infer, by extension, that Dungeness crab behavior, reproduction, and population densities may be altered or reduced by prolonged exposures to oiled sediments.

Phase 2

The low incidence of clasping and molting in all but the new control group was unexpected (Table 4). While no clasping and molting were exhibited in the new (4-month exposure) high dose and 75% of the crabs exhibited clasping and molting in the new controls, the incidence of these phenomena in the crabs held over from the long-term exposures (old control, old mid dose, old high dose) was quite low (8–16%). Clearly, exposure to oiled sediments for 14 months was not the lone cause of lack of clasping behavior and molting. Other stresses, such as confinement or lack of vital minerals, or both, and nutrients in their diet were probably operating in combination with exposure to the oil. The absence of molting in the new high dose (Table 4) does agree with findings of Karinen and Rice (1974), who found that brief (48 hours) exposure of premolt juvenile Tanner crabs to Prudhoe Bay crude oil inhibited molting. ”

It is generally believed that the reproductive biology of Dungeness crabs involves obligatory molting of the female before mating, and deposit of sperm in the spermatheca of the female while she is in a soft-shelled condition (Snow and Neilsen 1966; Wild 1983). Several months later, eggs are fertilized as they pass through the reproductive tract during spawning. Ebert et al. (1983) observed spawning in five crabs that had not molted; only one of those crabs oviposited a viable egg mass. We observed fresh sperm in spermathecas of crabs that were examined at the termination of Phase 2, which indicated that although the females had not molted, copulation had occurred,

The high incidence (55%) of extruded fertile eggs in females that had not molted suggests that the response to the combination of stressors (confinement, diet, and oiled sediments) is to use depleted energy reserves for spawning and forego the usual annual molting process.

CONCLUSION

The presence of oil in the substrate sediments of Dungeness crab habitat may lead to lower reproductive capability, lower viability of larvae, and, in combination with other stressors, inhibition of molting in adult female crabs. Over a period of time, these factors could reduce population levels in habitats affected by oil contamination.

REFERENCES CITED

Boolootian, R. A., A. C. Giese, A. Farmanfarmaian, and J. Tucker. 1959. Reproductive cycles of five West Coast crabs. Physiol. Zool. 32(4):213–220.

Brodersen, C. C., S. D. Rice, J. W. Short, T. A. Mecklenburg, and J. F. Karinen. 1977. Sensitivity of larval and adult Alaskan shrimp and crabs to acute exposures of the water-soluble fraction of Cook Inlet crude oil. Pages 575-578 *in 1977* Oil Spill Conference (Prevention, Behavior, Control, Cleanup) Proceedings. American Petroleum Institute, Environmental Protection Agency, and U.S. Coast Guard, Washington, D.C. 640 p.

Burns, K. A., and J. M. Teal. 1979. The West Falmouth oil spill: hydrocarbons in the salt marsh ecosystem. Estuar. Coast. Mar. Sci. 8:349–360.

Ebert, E. E., A. W. Haseltine, J. L. Houk, and R. O. Kelly. 1983. Laboratory cultivation of the Dungeness crab, *Cancer magister*. Pages 259–309 *in* P. W. Wild and R. N. Tasto (editors), Life History, Environment, and Mariculture Studies of the Dungeness Crab, *Cancer magister*, with Emphasis on the Central California Fishery Resource. Calif. Dep. Fish and Game, Fish Bull. 17'2.352 p.

Ebert, E. E., R. O. Kelly, and A. W. Haseltine. 1975. Observations on the larval hatching success of Dungeness crab, *Cancer magister*, from the San Francisco and Eureka-Crescent City regions. Calif. Dep. Fish and Game, Mar. Resour. Tech. Rep. No. 29. 14p.

Haines, J. R., and R. M. Atlas. 1982. *In situ* microbial degradation of Prudhoe Bay crude oil in Beaufort Sea sediments. Mar. Environ. Res. 7:91–102.

Karinen, J. F. 1980. Petroleum in the deep sea environment: potential for damage to biota. Environ. Internat. 3: 135–144.

Karinen, J. F., S. D. Rice, and M. M. Babcock. 1983. Long-term effects of petroleum hydrocarbons in sediment on reproduction and growth of crabs and shrimp in Alaska (specifically, *Chionoecetes bairdi*). Summary Report: L-RERP, P.L. 92-532, Sec. 202., Auke Bay Laboratory, Auke Bay, Alaska. 7 p.

Karinen, J. F., and S. D. Rice. 19'74. Effects of Prudhoe Bay crude on molting Tanner crabs, *Chionoecetes bairdi.* Mar. Fish. Rev. 36(7):31–37.

Krebs, C. T., and K. A. Burns. 1977. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax.* Science 197(4302):484–487.

MacKay, D., S. Paterson, P. D. Boehn, and D. L. Fiest. 1981. Physical-chemical weathering of petroleum hydrocarbons from the *Ixtoc I* blowout—chemical measurements and a weathering model. Pages 453-460 *in 1981 Oil* Spill Conference (Prevention, Behavior, Control, Cleanup) Proceedings. American Petroleum Institute, Environmental Protection Agency, and U.S. Coast Guard, Washington, D.C. '742 p.

Olla, B. L., W. H. Pearson, P. C. Sugarman, D. L. Woodruff, and J. W. Blaylock. 1981. The effects of petroleum hydrocarbons *on* chemoreception and behavior in the Dungeness crab, *Cancer magister.* EPA-600/7-81-093. 71 p.

Pearson, W. H., P. C. Sugarman, D. L. Woodruff, and J. W. Blaylock. 1980. Detection of petroleum hydrocarbons by the Dungeness crab, *Cancer magister.* Fish. Bull. 78(3):821–826.

Rice, S., J. F. Karinen, and C. C. Brodersen. 1983. Effects of oiled sediment on juvenile king crabs. OCSEAP Final Rep. 29:287–310.

Snow, C. D., and J. R. Neilsen. 1966. Premating and mating behavior of the Dungeness crab *(Cancer magister* Dana). J. Fish. Res. Bd. Can. 23(9):1319-1323.

Wild, P. W. 1983. The influence of seawater temperature on spawning, egg development, and hatching success of the Dungeness crab, *Cancer magister.* Pages 197–213 *in* P. W. Wild and R. N. Tasto (editors), Life History, Environment, and Mariculture Studies of the Dungeness Crab, *Cancer magister,* with Emphasis on the Central California Fishery Resource. Calif. Dep. Fish and Game, Fish Bull. 172.352 p.

APPENDIX

Egg Development: Dungeness Crab (Cancer *magister*)
(Adapted from Boolootian 1959.)

Stage	*Identifying Features*
0	Undivided cell.
1	2-128 cells: no yolk-free section apparent.
2	Yolk-free (transparent) section apparent; beginning of invagination.
3	Distinct division of egg into yolk and yolk-free sections; space develops between chorion and embryo.
4	Eye pigment barely apparent (individual pigment spots barely discernible under 40x).
5	Eye pigment more apparent: individual pigment spots seen at 40x.
6	Eyes strongly pigmented; much yolk still present (> 25% of egg volume); dorsal pigmentation becomes apparent.
7	Yolk reduced to two small patches,
8	No yolk present; prezoea stage recognizable.